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Coxiella burnetii/ Rickettsia prowazekii/Rickettsia typhi Multiplex Real Time PCR Kit User Manual

For Research Use Only

REF MBS598093 - Instrument III, IV

For use with ABI Prism®7000/7300/7500/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II; Bio-Rad CFX 6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler®480 Instrument



1. Intended Use

Coxiella burnetii/Rickettsia prowazekii/Rickettsia typhi Multiplex Real Time PCR Kit is used for the distinguishing of *Coxiella burnetii*, *Rickettsia prowazekii* and *Rickettsia typhi* in whole blood (non-anticoagulant), ticks, C.S.F. or skin tissue samples by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (C_t) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Rickettsiae are obligate intracellular parasites. They are the agents of typhus, spotted fever, and Q fever. The rickettsiae are a heterogeneous group of small, obligately intracellular, gram-negative coccobacilli and short bacilli, most of which are transmitted by a tick, mite, flea, or louse vector. Except in the case of louse-borne typhus, humans are incidental hosts. Among rickettsiae, *Coxiella burnetii*, *Rickettsia prowazekii*, and *Rickettsia typhi* have the well-documented ability to survive for an extended period outside the reservoir or vector and to be extremely infectious: inhalation of a single *Coxiella* microorganism can cause pneumonia.

Coxiella burnetii/Rickettsia prowazekii/Rickettsia typhi Multiplex real time PCR kit contains a specific ready-to-use system for the detection of *Coxiella burnetii*, *Rickettsia prowazekii* and *Rickettsia typhi* by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of 3 kinds of target genes. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. The detection of amplified *Coxiella burnetii* DNA fragment, *Rickettsia prowazekii* DNA fragment and *Rickettsia typhi* DNA fragment are performed in fluorimeter channel FAM, HEX /VIC/JOE and Cal Red610/ROX/TEXAS RED. DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the channel CY5 fluorescence of the internal control (IC). An external positive control contained.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	CB/RT Reaction Mix	1 vial, 950μl
3	PCR Enzyme Mix	1 vial, 12μl
4	Molecular Grade Water	1 vial, 400μl
5	CB/RT Internal Control(IC)	1 vial, 30μl
6	CB/RT Positive Control	1 vial, 30μl

Analysis sensitivity: 5×10^3 copies/ml;

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Trypsin digestive Solution
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 μl – 1000 μl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Tube racks

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area. • Avoid aerosols

8. Sample Collection, Storage and transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is contained in the kit, please thaw the buffer thoroughly and spin down briefly

in the centrifuge before use. You may use your own extraction systems or the commercial kit.

9.1.1 Whole blood, C.S.F, ticks samples

- Pipet 100μl sample to a 0.5ml tube, add 100μl DNA extraction buffer, close the tube and vortex for 10 seconds. Spin down briefly in a table centrifuge.
- Incubate the tube for 10 minutes at 100°C.
- Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and is used for PCR template.

9.1.2 Skin tissue samples

- Take 50mg sample to a tube, add 100μl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
- Incubate the tube for 10 minutes at 100°C.
- Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

Attention:

- During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination in case the sample is positive.
- The extraction sample should be used in 3 hours or store at -20°C for one month.
- DNA extraction kits are available from various manufacturers. You can also use your own extraction systems or the commercial kit depending on the yield. For DNA extraction, please comply with the manufacturer's instructions.

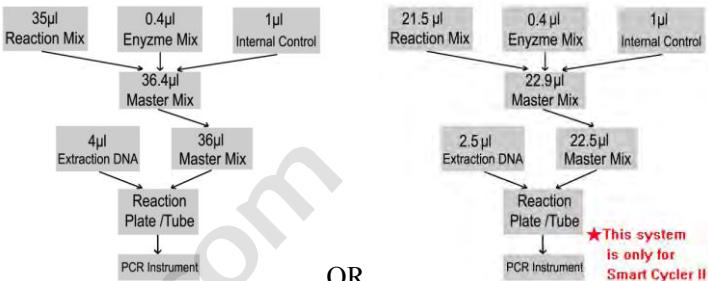
9.2 Internal Control

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.

Add the internal control (IC) 1μl/rxn and the result will be shown in the channel Cy5.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※PCR system without CY5 channel may be treated with 1μl Molecular Grade Water instead of 1μl IC.

- The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
- Pipet 36μl (22.5μl for Smart Cycler II)Master Mix with micropipets of sterile filter tips to each Real time PCR reaction plate/tubes. Separately add 4μl (2.5μl for Smart Cycler II)DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- Perform the following protocol in the instrument:

Selection of fluorescence channels	
FAM	<i>Coxiella burnetii</i>
HEX/VIC/JOE	<i>Rickettsia prowazekii</i>
Cal Red 610/ROX/TEXAS RED	<i>Rickettsia typhi</i>
CY5	IC

5) ▲If you use ABI Prism® system, please choose "none" as passive reference and quencher.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Quality control: Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Channel	Ct value			
	FAM	HEX	Cal Red 610	CY5
Control	UNDET	UNDET	UNDET	25~35
Molecular Grade Water	UNDET	UNDET	UNDET	25~35

12. Data Analysis and Interpretation : The following sample results are possible:

	FAM	HEX	Cal Red 610	CY5	Result Analysis
1#	UNDET	UNDET	UNDET	25~35	Below the detection limit or negative
2#	≤38	UNDET	UNDET	—	<i>Coxiella burnetii</i> positive;
3#	UNDET	≤38	UNDET	—	<i>Rickettsia prowazekii</i> positive;
4#	UNDET	UNDET	≤38	—	<i>Rickettsia typhi</i> positive;
5#	38~40			25~35	Re-test; If it is still 38~40, report as 1#
6#	UNDET				PCR Inhibition; No diagnosis can be concluded.

For further questions or problems,please contact our technical support _____